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(54) **GENE ET PROTEINE RELIES A LA MALADIE D'ALZHEIMER**
(54) **GENE AND PROTEIN RELATED TO ALZHEIMER'S DISEASE**

GENE AND PROTEIN RELATED TO ALZHEIMER'S DISEASE

Field of the Invention

The present invention relates to Alzheimer's Disease and, more particularly, to the identification of a gene and gene product associated with Alzheimer's Disease.

Background of the Invention

In order to facilitate reference to various journal articles, a listing of articles is provided at the end of the specification.

Wingless 1 (*Wnt1*) is a gene involved in signal transduction in D. melanogaster, Xenopus, rodents, human and many other vertebrates and invertebrates. The gene is involved in signaling during embryonic axis development, but it is also expressed in adult tissues. *Wnt1* exerts its effect through binding to the receptor *Drosophila frizzled 2* (*Dfz2*)[1], modulating the activity of *disheveled* [2], which suppresses the activity of the cytoplasmic serine/threonine kinase *zeste white 3* (also known as glycogen synthase kinase 3b- GSK3b) [3]. Inhibition of the phosphorylation of cytoplasmic proteins such as *armadillo* (also known as β-catenin) by inhibition of GSK3β causes the accumulation of these proteins (normal phosphorylated arm proteins are targeted for rapid proteasome mediated degradation) [3,4].

Description of the Invention

Genetic linkage studies have defined that a subset of pedigrees segregating familial Alzheimer Disease (FAD) but lacking mutations in the β-amyloid precursor protein gene (β APP), presenilin 1 or presenilin 2 genes (PS2), show cosegregation of the disease trait with genetic markers in the pericentromeric region of chromosome 12. The genetic markers D12S1057 and D12S1042 generate lod scores of $z \geq +3.00$ at low recombination factions and significant evidence for co-inheritance with an FAD trait using non-parametric statistics such as Affected Pedigree Member methods (M. Pericak-Vance, 11th International Symposium of the Tokyo Metropolitan Institute of Psychiatry, March 3rd, 1997 and Keystone Symposia on Molecular Mechanisms of Alzheimer Disease, Feb 1-6, 1997)

Several candidate genes exist in the vicinity of these genetic marker loci, including the human *Wingless 1* gene (hWnt1) whose biochemical properties would place it in biochemical pathways involving other FAD susceptibility genes such as βAPP and PS1.

A role for the *Wnt* pathway in Alzheimer's Disease (AD) is revealed by two observations. First, GSK3β is known to be the enzyme principally responsible for the hyperphosphorylation of the microtubule associated protein Tau in AD brain tissue [5]. Hyperphosphorylated Tau is in turn a principal component of the paired helical filament structures which form the Neurofibrillary Tangle, one of the major neuropathologic hallmarks of AD [6]. Secondly, another member of the *armadillo* protein family (GT24) has been shown to interact with PS1 (Levesque et al., in press). These observations suggest that mutations in the h*Wnt1* gene may be responsible for some cases of AD. These mutations might cause aberrant regulation of intracellular functions mediated by GSK3b (e.g. defective activation of the *Wnt* pathway causing suppression of GSK3β mediated hyperphosphorylation of Tau) or GT24 (e.g. aberrant regulation of the interaction of GT24 with PS1 and PS2 with resultant defects in the known biological activities of PS1 and PS2 such as abnormal sensitivity to apoptosis [7], aberrant processing of βAPP [8] and aberrant processing of the proteasome subunit S5a (Fraser et al., In Press). Evidence for a possible defect in GSK3β action in AD has been provided by biochemical and immunochemical assays showing increased immunoreactivity in AD brain tissue where it is expressed in appropriate cell types [5,9]. Other deleterious effects of persistent activation of GSK3β (also known as Tau Protein Kinase I - TPKI) include defects in energy metabolism and defects in choline acetyl transferase activity (which is another biochemical marker of AD) [5].

The fact that *Wnt1* is predominantly expressed in embryonic tissues and that deletion of this gene causes

severe embryonic defects in axial patterning does not preclude a role for mutations other than homozygous null mutants (i.e. knockouts) in adult onset neurodegeneration. Missense mutations in other embryonic signal transduction proteins important in axial patterning (i.e. Notch 3) have been associated with other adult onset neurodegenerative diseases such as CADASIL [10].

The genomic DNA sequence of the human Wnt1 gene has been determined by van Ooyen et al.,(23) and is shown in Table 1; the deduced amino acid sequence is shown in Table 2.

Primer sequences have been prepared, as described in Example 1, which permit the PCR amplification of each exon of the hWnt1 gene, allowing examination of the nucleotide sequence of the exons of this gene in a selected subject such as an AD patient. Amplified exons can be sequenced using standard methods and primers as described in Example 1.

GenOmic DNA from white blood cells of an FAD patient from the Tor 117 pedigree, associated with autopsy-proven FAD, was examined. A nucleotide substitution was found at nucleotide 1441 (nucleotide numbering as Table 1).

The T→A substitution in Exon 2 of the hWnt1 gene will lead to a non-conservative amino acid change, Ser88 being replaced by Arg.

The mutation co-segregates in the Tor 117 pedigre and is seen in four other AD affected members of the pedigree.

The identification of the association between FAD and the hWnt1 gene by the present inventors enables numerous applications.

In one series of embodiments, this invention provides primers complementary to the Wnt1 gene sequence which may be used to identify mutations causing AD, as exemplified by the identification of the missense mutation described in Example 1.

In accordance with another aspect of the invention, a recombinant vector for transforming a mammalian or invertebrate tissue cell to express a normal or mutant Wnt1 sequence in the cells is provided.

In another series of embodiments, the present invention provides for host cells which have been transfected or otherwise transformed with the nucleotide sequence of the Wnt1 gene. The cells may be transformed merely for purposes of propagating an inserted nucleic acid construct, or may be transformed so as to express the normal or mutant Wnt1 protein. The transformed cells of the invention may be used in assays to identify proteins and/or other compounds which affect normal or mutant Wnt1 expression, which interact with the normal or mutant Wnt1 proteins, and/or which modulate the function or effects of the normal or mutant proteins, or to produce Wnt1 proteins, fusion proteins, functional domains, antigenic determinants, and/or antibodies to the Wnt1 protein.

Transformed cells may also be implanted into hosts, including humans, for therapeutic or other reasons. Preferred host cells include mammalian cells from neuronal, fibroblast, bone marrow, spleen, organotypic or mixed cell cultures, as well as bacterial, yeast, nematode, insect and other invertebrate cells. For uses as described below, preferred cells also include embryonic stem cells, zygotes, gametes, and germ line cells.

In another series of embodiments, the present invention provides transgenic animal models for AD and other diseases or disorders associated with mutations in the Wnt1 gene. The animal may be essentially any mammal, including rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates. In addition, invertebrate models, including nematodes and insects, may be used for certain applications. The animal models are produced by standard transgenic methods

including microinjection, transfection, or other forms of transformation of embryonic stem cells, zygotes, gametes, and germ line cells with vectors including genomic or cDNA fragments, minigenes, homologous recombination vectors, viral insertion vectors and the like. Suitable vectors include vaccinia virus, adenovirus, adeno associated virus, retrovirus, liposome transport, neuraltropic viruses, and Herpes simplex virus. The animal models may include transgenic sequences comprising or derived from the Wnt1 gene, including normal and mutant sequences, intronic, exonic and untranslated sequences, and sequences encoding subsets of the Wnt1 protein, such as functional domains. The major types of animal models provided include: (1) Animals in which a normal human Wnt1 gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; and animals in which a normal human Wnt1 gene has been recombinantly substituted for one or both copies of the animal's homologous Wnt1 gene by homologous recombination or gene targeting; and/or in which one or both copies of one of the animal's homologous Wnt1 genes have been recombinantly "humanized" by the partial substitution of sequences encoding the human homologue by homologous recombination or gene targeting . (2) Animals in which a mutant human Wnt1 gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; and animals in which a mutant human Wnt1 gene has been recombinantly substituted for one or both copies of the animal's homologous Wnt1 gene by homologous recombination or gene targeting; and/or in which one or both copies of one of the animal's homologous Wnt1 genes have been recombinantly "humanized" by the partial substitution of

sequences encoding a mutant human homologue by homologous recombination or gene targeting. (3) Animals in which a mutant version of one of that animal's Wnt1 genes has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; and/or in which a mutant version of one of that animal's Wnt1 genes has been recombinantly substituted for one or both copies of the animal's homologous Wnt1 gene by homologous recombination or gene targeting. (4) "Knock-out" animals in which one or both copies of one of the animal's Wnt1 genes have been partially or completely deleted by homologous recombination or gene targeting, or have been inactivated by the insertion or substitution by homologous recombination or gene targeting of exogenous sequences. In preferred embodiments, a transgenic mouse model for AD has a transgene encoding a normal human Wnt1 protein, a mutant human or murine Wnt1 protein, or a humanized normal or mutant murine Wnt1 protein.

The present invention also specifically provides for mutant or disease-causing variants of the human Wnt1 protein by disclosing a specific mutant sequences and by providing methods by which other such variants may be routinely obtained. Because the Wnt1 proteins may be used in a variety of diagnostic, therapeutic and recombinant applications, various subsets of the Wnt1 protein sequence and combinations of the Wnt1 protein sequence with heterologous sequences are also provided. For example, for use as immunogens or in binding assays, subsets of the Wnt1 protein sequence, including both normal and mutant sequences, are provided. Such protein sequences may comprise a small number of consecutive amino acid residues from the sequence disclosed herein but preferably include at least 4-8, and preferably at least 9-15 consecutive amino acid residues from a Wnt1 sequence. Other preferred subsets of the Wnt1 protein

sequence include those corresponding to one or more of the functional domains or antigenic determinants of the Wnt1 protein and, in particular, may include either normal (wild-type) or mutant sequences. The invention also provides for various protein constructs in which Wnt1 sequence, either complete or subsets, is joined to exogenous sequences to form fusion proteins and the like.

In another series of embodiments, the present invention provides for the production and use of polyclonal and monoclonal antibodies, including antibody fragments, including Fab fragments, $F(ab')_2$, and single chain antibody fragments, which selectively bind to the Wnt1 protein, or to specific antigenic determinants of the protein for use in various diagnostic, therapeutic and technical applications described herein. The antibodies may be raised in mouse, rabbit, goat or other suitable animals, or may be produced recombinantly in cultured cells such as hybridoma cell lines. Preferably, the antibodies are raised against Wnt1 sequences comprising at least 4-8, and preferably at least 9-15 consecutive amino acid residues from the Wnt1 sequence.

In another series of embodiments, the present invention provides methods of screening or identifying proteins, small molecules or other compounds which are capable of inducing or inhibiting the expression of the hWnt1 gene. The assays may be performed <u>in vitro</u> using non-transformed cells, immortalized cell lines, or recombinant cell lines, or <u>in vivo</u> using the transgenic animal models enabled herein. In particular, the assays may detect the presence of increased or decreased expression of *hWnt1* or other Wnt1-related genes or proteins on the basis of increased or decreased mRNA expression, increased or decreased levels of presenilin-related protein products, or increased or decreased levels of expression of a marker gene (e.g., β-galactosidase, green fluorescent protein, alkaline phosphatase or luciferase) operably joined to a Wnt1 5'

regulatory region in a recombinant construct. Cells known to express a particular Wnt1, or transformed to express Wnt1, are incubated and one or more test compounds are added to the medium. After allowing a sufficient period of time (e.g., 0-72 hours) for the compound to induce or inhibit the expression of the *hWnt1* gene, any change in levels of expression from an established baseline may be detected using any of the techniques described above. In particularly preferred embodiments, the cells are from an immortalized cell line such as a human neuroblastoma, glioblastoma or a hybridoma cell line, or are transformed cells of the invention.

In another series of embodiments, the present invention provides methods for identifying proteins and other compounds which bind to, or otherwise directly interact with, hWnt1 protein. The proteins and compounds will include endogenous cellular components which interact with the Wnt1 protein in vivo and which, therefore, provide new targets for pharmaceutical and therapeutic interventions, as well as recombinant, synthetic and otherwise exogenous compounds which may have Wnt1 protein binding capacity and, therefore, may be candidates for pharmaceutical agents. Thus, in one series of embodiments, cell lysates or tissue homogenates (e.g., human brain homogenates, lymphocyte lysates) may be screened for proteins or other compounds which bind either to normal or to mutant Wnt1 protein. Alternatively, any of a variety of exogenous compounds, both naturally occurring and/or synthetic (e.g., libraries of small molecules or peptides), may be screened for Wnt1 binding capacity. In each of these embodiments, an assay is conducted to detect binding between a "Wnt1 component" and some other moiety. The "Wnt1 component" in these assays may be any polypeptide comprising or derived from a normal or mutant Wnt1 protein, including functional domains or antigenic determinants of the Wnt1 protein, or Wnt1 fusion

proteins. Binding may be detected by non-specific measures (e.g., changes in intracellular Ca^{2+}, GTP/GDP ratio) or by specific measures (e.g., changes in Ab peptide production or changes in the expression of other downstream genes which can be monitored by differential display, 2D gel electrophoresis, differential hybridization, or SAGE methods). The preferred methods involve variations on the following techniques: (1) direct extraction by affinity chromatography; (2) co-isolation of Wnt1 components and bound proteins or other compounds by immunoprecipitation; (3) the Biomolecular Interaction Assay (BIAcore); and (4) the yeast two-hybrid systems.

The identification of other cellular proteins which interact with the hWnt1 protein, as described above, enables the identification of other genes involved in the biochemical pathway causing AD.

Alternatively, tissues, fluids or cells of AD-affected or at-risk subjects can be used for the analysis of the DNA sequence, transcriptional pattern, protein expression, protein post-translational modification (phosphorylation etc), and biochemical/functional activity of other genes known to function in the same biochemical pathways as these genes. Thus, for *Wnt1*, these other genes would include other members of the mammalian *Wingless* family of genes, other genes in the known *Wingless* signalling pathways (e.g. *frizzled* receptors, *dishevelled* homologues, glycogen synthetase kinase 3β, other armadillo proteins), and other genes known to be involved in process of wingless (e.g. mammalian homologues of *porcupine*[11]).

In another series of embodiments, the present invention provides for methods of identifying proteins, small molecules and other compounds capable of modulating the activity of normal or mutant Wnt1 protein. Using normal cells or animals, the transformed cells and transgenic animal models of the present invention, or

cells obtained from subjects bearing normal or mutant *hWnt1* genes, the present invention provides methods of identifying such compounds on the basis of their ability to affect the expression of theWnt1, the intracellular localization of Wnt1 protein, intracellular Ca^{2+}, Na^{+}, K^{+} or other ion levels or metabolism, the occurrence or rate of apoptosis or cell death, the levels or pattern of Ab peptide production, the presence or levels of phosphorylation of microtubule associated proteins, or other biochemical, histological, or physiological markers which distinguish cells bearing normal and mutant Wnt1 sequences. Using the transgenic animals of the invention, methods of identifying such compounds are also provided on the basis of the ability of the compounds to affect behavioral, physiological or histological phenotypes associated with mutations in Wnt1 protein.

In another series of embodiments, the present invention provides methods for screening for carriers of *Wnt1* alleles associated with AD, for diagnosis of victims of AD, and for the screening and diagnosis of related presenile and senile dementias, psychiatric diseases such as schizophrenia and depression, and neurologic diseases such as stroke and cerebral hemorrhage, which associated with mutations in the Wnt1 gene. Screening and/or diagnosis can be accomplished by methods based upon the nucleic acids, proteins, and/or antibodies disclosed and enabled herein, including functional assays designed to detect failure or augmentation of the normal Wnt1 activity and/or the presence of specific new activities conferred by the mutant Wnt1 protein. Thus, screens and diagnostics based upon Wnt1 proteins are provided which detect differences between mutant and normal Wnt1 in electrophoretic mobility, in proteolytic cleavage patterns, in molar ratios of the various amino acid residues, in ability to bind specific antibodies. In addition, screens and diagnostics based upon nucleic acids and primers are provided which detect differences

in nucleotide sequences by direct nucleotide sequencing, hybridization using allele specific oligonucleotides, restriction enzyme digest and mapping (e.g., RFLP. REF-SSCP), electrophoretic mobility (e.g., SSCP, DGGE), PCR mapping, RNase protection, chemical mismatch cleavage, ligase-mediated detection, and various other methods. In accordance with these embodiments, diagnostic kits are also provided which will include the reagents necessary for the above-described diagnostic screens.

In another series of embodiments, the present invention provides methods and pharmaceutical preparations for use in the treatment of Wnt1-associated diseases such as AD. These methods and pharmaceuticals are be based upon (1) administration of normal Wnt1 proteins, (2) gene therapy with a normal Wnt1 gene to compensate for or replace a mutant gene, (3) gene therapy based upon antisense sequences to a mutant Wnt1 gene or which "knock-out" the mutant gene, (4) gene therapy based upon sequences which encode a protein which blocks or corrects the deleterious effects of Wnt1 mutants, (5) immunotherapy based upon antibodies to normal and/or mutant Wnt1 proteins, or (6) small molecules (drugs) which alter Wnt1 expression, block abnormal interactions between mutant forms of Wnt1 and other proteins or ligands, or which otherwise block the aberrant function of mutant Wnt1 proteins by altering the structure of the mutant proteins, by enhancing their metabolic clearance, or by inhibiting their function.

The activity of *hWnt1* can be manipulated pharmacologically by several mechanisms including drugs which promote or inhibit post-translational modification of the Wnt1 protein (e.g. phorbol esters okadaic acid to alter phosphorylation state, inhibitors of glycosylation etc.).

The wingless signalling pathway is well worked out in D. melanogaster, Xenopus, rodents and many other

invertebrate and vertebrate animals. These systems can be used to devise means of altering the activity of mutant or wild type wingless signalling pathways (ie wingless and its downstream partners including glycogen synthase kinase 3 beta)in humans with AD regardless of whether AD arises as a result of mutations in wingless or arising through other mechanisms. Such modulating mechanisms would include the following items.

Wnt signalling pathways can be modulatd by Lithium (24), by protein kinase C inhibitors such as Ro31-8220 as well as phorbol esters (25), as well as by decapentaplegic protein (24) and Notch (27) either by directly influencing Wingless express or by influencing downstream elements in the wingless signaling pathway. These, or analogues or antagonists could be used to modulate wingless signaling (and relevant downstream events such as glycogen synthase kinase 3-beta (GSK-3b)activation in patients with AD and can be used as starting points for drug design.

Because *Wnt1* binds to specific proteins and/or receptor-like molecules (*Wnt1* binds to *fizzled* receptors[1]) these interactions can be modelled and used to develop selective antagonists, agonist, competitive inhibitors or competitive agonists which possess selective activities against mutant isoforms of these proteins.

The sequences can be used to identify other genes involved in the biochemical pathway causing AD by employing techniques such as yeast-two-hybrid methods to identify other cellular proteins interacting with the respective protein. Alternatively, tissues. fluids or cells of AD affected or at-risk subjects can be used for the analysis of the DNA sequence, transcriptional pattern, protein expression, protein post-translational modification (phosphorylation etc), and biochemical/functional activity of other genes known to

function in the same biochemical pathways as these genes. Thus, for *Wnt1* these other genes would include other members of the mammalian *Wingless* family of genes, other genes in the known *Wingless* signaling pathways (e.g. *frizzled* receptors, *dishevelled* homologues, glycogen synthetase kinase 3β, other armadillo proteins), and other genes known to be involved in processing of wingless (e.g. mammalian homologues of *porcupine* [11]).

The nucleotide sequences and/or proteins can themselves be used as direct therapeutic agents. Dominant loss of function mutations (constitutively inactive) and dominant gain of function mutations (constitutively active) have been described in *Wnt1*. Thus if disease causing mutations in these genes have a dominant negative effect, appropriate nucleotides or recombinant proteins can be made with a countervailing dominant gain of function activities and administered via several routes such as protein infusion (*Wnt1* is a soluble extracellular molecule) or transfection (using vectors such as H. simplex).

Assays exist for the *Wnt1* gene (e.g. transformation of C57MG mammary epithelial cell lines[12], myogenesis in somites and segmental plates co-cultured with *Wnt1*[13], biochemical assays of *armadillo* levels in cultured Drosophila cl-8 imaginal disc cell lines[14]). These *in vitro* assays can be used to judge the effect of the FAD related missense mutations and to screen for drugs which act at *Wnt1* itself, at upstream, or at downstream sites in the *Wnt* signaling pathways, and which might be used to counteract the effect of the *Wnt1* mutations.

In accordance with another aspect of the invention, the proteins of the invention can be used as starting points for rational drug design to provide ligands, therapeutic drugs or other types of small chemical molecules. Alternatively, small molecules or other

compounds identified by screening assays may serve as "lead compounds" in rational drug design.

Example 1

Genomic sequences for each exon of *hWnt1* can be amplified using primers 1306 (5'-AGCCTCCTCCCGTCACTTCAG) and 1307 (5'-GGATCATTCGCCCCACTTGTA) for Exon 1; 1308 (5'-CTGGGAGAGCGGGTATTATTA) and 1309 (5'CTGGGCACGAGGCACTTGG) for Exon 2: and 1310 (5'-CTGCTCCACTTCCGCTATCG) and 1311 (5'-TGCCCCTTGCCTTATCTCAC) for Exon 3; and 1312 (5'-CCTGAGAGGCCGAGACTGACT) and 1313 (5'-GGAGAGATGGGATGCGTATGAA) for Exon 4. PCR conditions for these primers are Exon 1: 100ng genomic DNA, $MgCl_2$ 1.5 mM, dNTPs 250 mM, primers 50 pmol, Taq polymerase 0.5 Units, DMSO 5% in reaction volume of 50 ul with thermocycles of 94°C X 30secs, 58°C X 20secs, 72°C X 30secs, for 34 cycles. Exon 2: as for Exon 1 except $MgCl_2$ 2.5 mM, DMSO 10% and annealing temperature of 60°C. Exon 3: as for Exon 1 except $MgCl_2$ 2.5 mM, no DMSO, annealing temperature of 58°C. Exon 4: as for Exon 1 except $MgCl_2$ 1.5 mM, DMSO 5%, and annealing temperature of 59°C.

These exons can then be sequenced using standard dideoxy-cycle sequencing methods employing primers 1316 (5'-CGGGCAACAACCAAAGTC) for Exon 1; 1317 (5'-CGGGTGGCACAGTTTTTA) for Exon 2; 1318 (5'-CCCCTTGCCTTATCTCAC) for Exon 3; 1319 (5'-CCGGGAGAGGGCAGTGTC) and 1327 (5'-AACCGGGTCTTGAGTGCT) for Exon 4.

Analysis of the genomic sequences for Exons 1-4 of *hWnt1* detected a T→A substitution at nucleotide 1441 (Figure 1: Accession #:X03072) in Exon 2 coding sequence which results in the non-conservative substitution of Ser88 by Arg (*hWnt1* Ser88Arg) in an affected member of a pedigree (Tor117) segregating autopsy-proven Familial Alzheimer Disease. Ser88 is conserved in *Wnt1* sequences

in vertebrates above birds and in Xenopus, and is conservatively substituted by Ala or Thr in some lower vertebrates, and by Ala, Leu, Iso, or Thr in most other *Wnt* homologues such as *Wnt3*, *Wnt3a*, *Wnt5*, *Wnt5a*.

Table 1

Accession # X03072 (G DB genome)

ORIGIN

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    1 cagctgagtg aggcgggcgc gcgtgggagg gtgtcccaag gggaggggtc cgcggccagt
   61 gcaggcccgg aggcgggggc caccgggcag ggggcggggg tgagccccga cggccaaccc
  121 gtcagctctc ggctcagacg ggcgggaacc acagccccgc tcgctgccca ttgtctgcgc
  181 ccctaaccgg tgcgccctgg tgccacagtg cggcccggag gggcagcctc ctcccgtcac
  241 ttcagccagc gccgcaacta taagaggcgg tgccgcccgc cgtggccgcc tcagcccacc
  301 agccgggacc gcgagccatg ctgtccgccg cccgccccca gggttgttaa agccagactg
  361 cgaactctcg ccactgccgc caccgccgcg tcccgtccca ccgtcgcggg caacaaccaa
  421 agtcgccgca actgcagcac agagcgggca aagccaggca ggccatgggg ctctgggcgc
  481 tgttgcctgg ctgggtttct gctacgctgc tgctggcgct ggccgctctg cccgcagccc
  541 tggctgccaa cagcagtggc cgatggtggt aagtgagctg gtgcggggtc gccacttgtc
  601 ccgcggcaca gagccagggg ccaaccctac ccagctccca cgctctggga tccgtctgcc
  661 gacaggctcc ctccccgctc tgacttccct ccgcgacacc gaagggcgat ctggcatgaa
  721 actgccccag actccagctc tgtacaagtg gggcgaatga tccgcccgcg gaggcctaag
  781 ataccccagg cagggagccc actctcatct agcaccgccc ttccccttta agcgccaact
  841 ccagcctcac ggcggtggct caccacaggt ttccccacct cgggaagtga agggccagga
  901 gttcgcctag aaaggagggg agaagagggt gggactccta agcatttcac gccttgggtg
  961 ggcaagaact gcaggccatg attatctcgc tcaggctgac cggaagaggc tcggagatcc
 1021 aaggtagaca ctcggtctcc gggtacctcc tctgtccagt ctccggacct agggctcagg
 1081 cgagcagccc tgggactact gggcacacac aagtctggac gcccagttct ttcaaattag
 1141 tgagcctggg agagcgggta ttattaatct cccgccattc tctccagcca catacccccа
 1201 ggaagaggac cgggtggcac agtttttatg gttagggtgc ggatcccctt cctgagcctg
 1261 agctatcata cgtcccacca ggggtattgt gaacgtagcc tcctccacga acctgcttac
 1321 agactccaag agtctgcaac tggtactcga gcccagtctg cagctgttga gccgcaaaca
 1381 gcggcgcctg atacgccaaa atccggggat cctgcacagc gtgagtgggg ggctgcagag
 1441 tgccgtgcgc gagtgcaagt ggcagttccg gaatcgccgc tggaactgtc ccactgctcc
 1501 agggccccac ctcttcggca agatcgtcaa ccgaggtggg tgcccaggaa ggcgacgctt
 1561 ccgggagcag gggaaacgcg gggtcacccc cagggcatgg gcgggcgagt tcagagaagg
 1621 tgtcccaggc gcctggaggg tcacacaatc aaccttgcca agtgcctcgt gcccagcgcc
 1681 agctcggggc cagacttcta ccaggcgttt tccagccgtg caccctggaa acgaagctta
 1741 acttttctga gctactgccc cagataaaga aagtttcggg tcgcggacgc cggctgaccg
 1801 ccgctttccc ccagcctctc tcaaaagcgc ctgggaagct gctctctgca ggcgtgtgtc
 1861 tggcctctcg cccagcaagg cttgcaccgc caaaatgggc cgaaagtttt gggctgcgaa
 1921 gaagtcttgg ggatgtatgg ttcttccgct cccctctctt cggtttgtct ctctggggct
 1981gctccacttc cgctatcgag ccaaaatgcg ccctagaatc tcccagtaag gtgtgattac
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Table 1 Continued

2041 gcccgtggac gtggctgcgt gcccacgcac ctgctttctc tactagccct agagaccagc
2101 tttccagcac tgccggccct ggtcctcagg actcaaagtg cggagtcggg ggtgggattc
2161 cggtcccaag cccttcatga gggtgctggc cgcgccccgc gtacccccctc gctgatcccc
2221 gctcccttct cccacaggct gtcgagaaac ggcgtttatc ttcgctatca cctccgccgg
2281 ggtcacccat tcggtggcgc gctcctgctc agaaggttcc atcgaatcct gcacgtgtga
2341 ctaccggcgg cgcggccccg ggggccccga ctggcactgg gggggctgca gcgacaacat
2401 tgacttcggc cgcctcttcg gccgggagtt cgtggactcc ggggagaagg ggcgggacct
2461 gcgcttcctc atgaaccttc acaacaacga ggcaggccgt acggtgagct ttgagaggct
2521 ccgcacccta agcggagcgg caggggccaa cctcgggctg gggaagtgac ggtcggtgag
2581 ataaggcaag gggcaccagg agagggcgtc ctgggagagc cggaggcttg gaacgaagac
2641 ggagaataga ggagacagtg gctgagggca aaggtatgtc tggcccgcgg acaggtagaa
2701 gaggttgcaa atcaagcaca gtctcttcgc tgtacagatt cgaaaaataa gcctgagagg
2761 ccgagactga ctcgccgcgg cggagcaggg ttgggcaggg tttccaaatc tcagcggaac
2821 atttcgcgcc tcccttcccc tgggctcagc taggcctggg cctttgctga ggtccggccc
2881 ccgtggcgtc cgggagaggg cagtgtctgg gagggtgact ctggcccggt gccctgggac
2941 actctttctt cccctatccc cgcagaccgt attctccgag atgcgccagg agtgcaagtg
3001 ccacgggatg tccggctcat gcacggtgcg cacgtgctgg atgcggctgc ccacgctgcg
3061 cgccgtgggc gatgtgctgc gcgaccgctt cgacggcgcc tcgcgcgtcc tgtacggcaa
3121 ccgcggcagc aaccgcgctt cgcgagcgga gctgctgcgc ctggagccgg aagacccggc
3181 ccacaaaccg ccctcccccc acgacctcgt ctacttcgag aaatcgccca acttctgcac
3241 gtacagcgga cgcctgggca cagcaggcac ggcagggcgc gcctgtaaca gctcgtcgcc
3301 cgcgctggac ggctgcgagc tgctctgctg cggcaggggc caccgcacgc gcacgcagcg
3361 cgtcaccgag cgctgcaact gcaccttcca ctggtgctgc cacgtcagct gccgcaactg
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Table 1 Continued

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4321 gatgcgtttg cctcctggaa tcagtatttc cttccactgt agctattagc ggctcctcgc
4381 ccccaccagt gtagcatctt cctctgcaga ataaaatctc tatttttatc gatgacttgg
4441 tggcttttcc ttgaatccag aacacaacct tgtttgtggt gtcccctatc ctccccttt
4501 accactccca gcttggaagc tt

LOCUS      HSINT1G      4522 bp    DNA    PRI  03-JAN-1991
DEFINITION  Human int-1 mammary oncogene.
ACCESSION   X03072
NID         g33935
KEYWORDS    int-1 oncogene; oncogene.
SOURCE      human.
ORGANISM    Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa;
            Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini;
            Hominidae; Homo.
REFERENCE   1  (bases 1 to 4522)
AUTHORS     van Ooyen,A., Kwee,V. and Nusse,R.
TITLE       The nucleotide sequence of the human int-1
            mammary oncogene;
            evolutionary conservation of coding and non-
            coding sequences
JOURNAL     EMBO J. 4 (11), 2905-2909 (1985)
MEDLINE     86055728
COMMENT     Data kindly reviewed (15-JUN-1986) by R. Nusse.
FEATURES    Location/Qualifiers
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promoter     259..263
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                        /db_xref="PID:g33936"
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Table 1 Continued

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     intron     1536..2237
                /note="intron II"
     intron     2504..2965
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                /note="pot. polyadenylation signal"
     BASE COUNT      805 a   1523 c   1320 g    874 t
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Table 2

MTSRSTARPNGQPQASKICQFKLVLLGESAVGKSSLVLRFVKGQ
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TNQETFARAKTWVKELQRQASPSIVIALAGNKADLANKRMVEYEEAQAYADDNSLLFM
ETSAKTAMNVNDLFLAIAKKLPKSEPQNLGGAAGRSRGVDLHEQSQQNKSQCCSN

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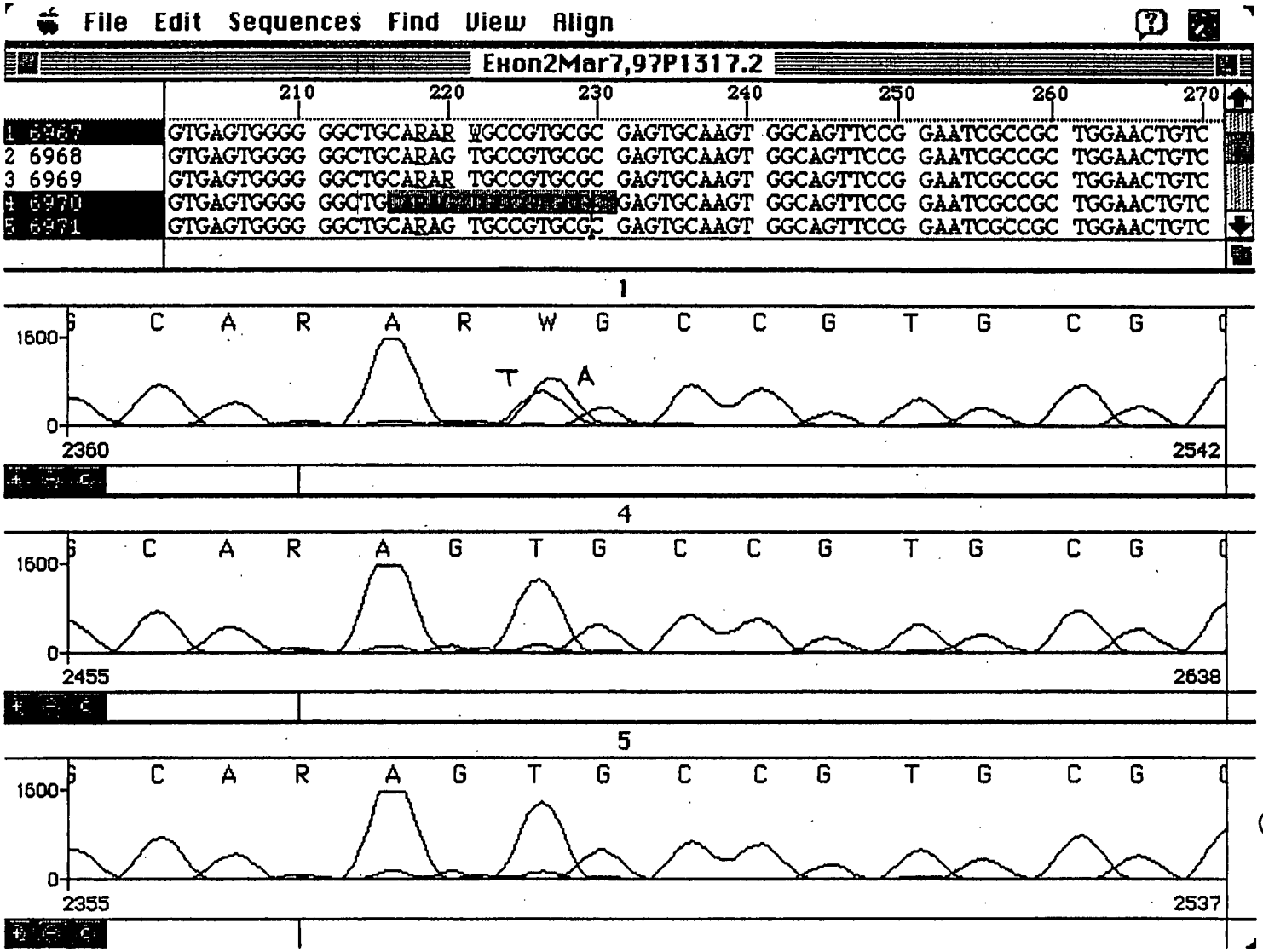

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